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16. ABSTRACT

Granulin and polyhefrin proteins were purified by preparative sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis from the baculoviruses Autographa californica, Rachiplusia ou, Heliothis zea, Heliothis armigera, Trichoplusia ni, and Spodotera frugiperda. Antisera were raised against Autographa californica (Ac) polyhedrin and Trichoplusia ni (Tn) granulin and analyzed for homologous and heterologous immunoreactivity by immunodiffusion and radioimmunoassay (RIA). Ac polyhedrin and Tn granulin antisera recognized antigenic determinants on several baculovirus polyhedrin and granulin proteins even though the heterologous proteins had different immunoreactivities when compared by competition radioimmunoassay. Antigenic differences among granulin and polyhedrin proteins were also detected by altered slopes of the competition reaction curves. Antiserum raised against Ac polyhedrin which was purified in the presence of SDS was tested by competition RIA for its ability to detect and react with native polyhedrin produced in the infected TN-368 cells. Ac polyhedrin antiserum had similar if not identical ability to bind to native polyhedrin and to polyhedrin purified in the presence of SDS.

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Radioimmunoassay Analysis of Baculovirus Granulins and Polyhedrins

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Granulin and polyhedrin proteins were purified by preparative sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis from the baculoviruses *Autographa californica*, *Rachiplusia ou*, *Heliothis zea*, *Heliothis armigera*, *Trichoplusia ni*, and *Spodoptera frugiperda*. Antisera were raised against *Autographa californica* (Ac) polyhedrin and *Trichoplusia ni* (Tn) granulin and analyzed for homologous and heterologous immunoreactivity by immunodiffusion and radioimmunoassay (RIA). Ac polyhedrin and Tn granulin antisera recognized antigenic determinants on several baculovirus polyhedrin and granulin proteins even though the heterologous proteins had different immunoreactivities when compared by competition radioimmunoassay. Antigenic differences among granulin and polyhedrin proteins were also detected by altered slopes of the competition reaction curves. Antiserum raised against Ac polyhedrin which was purified in the presence of SDS was tested by competition RIA for its ability to detect and react with native polyhedrin produced in the infected TN-368 cells. Ac polyhedrin antiserum had similar if not identical ability to bind to native polyhedrin and to polyhedrin purified in the presence of SDS.

INTRODUCTION

Baculoviruses are complex both in physical structure and protein composition and they contain large, covalently closed DNA genomes (Smith and Summers, 1979). The potential use of baculovirus genomes as cloning vectors (*Fed. Reg.* 43, 33043–33178), and the use of baculovirus polyhedra as microbial pesticides (Summers *et al.*, 1975; Summers and Kawanishi, 1978) are areas of research and application which require more studies on viral genetics and the nature of host range specificity. We published physical maps of wild and plaque-purified isolates of *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) DNAs (Smith and Summers, 1978, 1979), and characterized the structural polypeptide composition of the Ac*M*NPV enveloped nucleocapsid and polyhedrin relative to other baculoviruses (Summers and Smith, 1978). The development and application of sensitive and specific serological techniques such as radioimmunoassay (RIA) would provide another useful tool for identifying viral gene products and studying their functional roles in invasion and infection.

Baculovirus enveloped nucleocapsids are embedded in a paracrystalline inclusion which is composed of a single major structural polypeptide called polyhedrin for nuclear polyhedrosis viruses (NPVs) and granulin for granulosis viruses (GVs) (Summers and Egawa, 1973). These proteins vary in molecular weight from 25,000 to 31,000 and as detected by immunodiffusion (Crozier and Meynadier, 1972a, b, 1973, 1975; Norton and DiCapua, 1976; Rohrmann *et al.*, 1977) and radioimmunoassay (Crawford *et al.*, 1977, 1978; Crawford and Kalmakoff, 1977) share cross-reacting antigenic determinants. Tryptic peptide analysis (Summers and Smith, 1976) of several baculovirus polyhedrin and granulin proteins shows that similar tryptic peptides are present, suggesting that some primary structure is conserved within these proteins thus providing a partial explanation for the serological cross-reactions. However, each polyhedrin and granulin compared has tryptic peptides unique for that virus. This provides support for classifying granulins and polyhedrins as group-related proteins and supports the hypothesis that they are coded for by the viral genome (Summers and Smith, 1976; Maruniak and Summers, 1978).

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In this report, we described the efficacy of preparative polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) for isolating granulin and polyhedrin proteins for use in immunological studies. Using antiserum prepared against SDS-purified polyhedrin and granulin we used competition RIA to assay antigenicity and to compare the immunoreactivity of SDS-purified Ac*M* NPV polyhedrin and polyhedrin synthesized in infected cells.

MATERIALS AND METHODS

Viruses. The baculoviruses utilized in this investigation were: *Autographa californica* (Ac*M* NPV), *Rachiplusia ou* (Ro*M* NPV), *Heliothis armigera* (Ha*M* NPV), *Heliothis zea* (*Hz*S NPV), *Trichoplusia ni* (Tn*S* NPV) nuclear polyhedrosis viruses and *Trichopulsia ni* (TnGV) and *Spodoptera frugiperda* (SfGV) granulosis viruses. The sources of viruses and methods of handling were reported previously (Summers and Smith, 1976, 1978).

Cell culture. The TN-368-10 cell line used in this study was cloned from TN-368 cells (Volkman and Summers, 1975, 1976) and was maintained in continuous culture at 28° in TNM-FH medium (Hink, 1970).

Purification of polyhedrins and granulins. Polyhedra from infected insects were purified by centrifugation and banding at buoyant density in 40–63% (wt%) continuous sucrose gradients as previously reported (Summers and Smith, 1976). The purified polyhedra were suspended in 0.01 *M* Tris (pH 7.8), 0.01 *M* EDTA at a final concentration of 5.0 mg protein/ml and incubated at 70–72° for 2 hr. To further insure inactivation of the associated protease $HgCl_2$ was added to a final concentration of 0.01 *M* and equilibrated overnight at room temperature. To remove buffer and inhibitor the treated polyhedra were washed extensively by suspending the polyhedra in water and pelleting by differential centrifugation. Polyhedra were solubilized by adding 0.1 *M* Na_2CO_3, 0.17 *M* NaCl, pH 10.8, for 10 min (5 mg protein/ml) at 4°. The alkali-solubilized preparation was centrifuged at 24,000 rpm (SW 27 rotor) for 20 min at 4° to remove the insoluble material. The supernatant was made 1.0% with respect to SDS, 5.0% with 2-mercaptoethanol, and boiled for 3 min. The polyhedrins or granulins were electrophoresed in preparative vertical slab gels in the presence of 0.1% SDS. Following electrophoresis, the portion of the gel slab containing the polyhedrin or granulin was removed and the protein extracted from the polyacrylamide as described (Summers and Smith, 1975, 1976). The extracted proteins were analyzed for purity and structural integrity by analytical polyacrylamide gel electrophoresis in the presence of 0.1% SDS (Laemmli, 1970; Summers and Smith, 1978), then stored at −90°. For autoradiography gel slabs were dried onto filter paper and exposed to Kodak X-ray film for 48 hr.

Radioiodination of granulin and polyhedrin. Ac*M* NPV polyhedrin and TnGV granulin were labeled with carrier-free $Na^{125}I$ (New England Nuclear) by the chloramine-T method (Greenwood *et al.*, 1963). ^{125}I-Protein was separated from free ^{125}I by chromatography using a P-60 (Bio-Rad) column. The labeled protein was stored at −90° in 0.01% sodium azide (NaN_3), and used within 2 weeks. A progressive loss in immunoreactivity occurred upon extended storage at −90°, storage at 4°, and labeling to greater than 20 μCi/μg protein.

Antisera. Antisera to Ac*M* NPV polyhedrin and TnGV granulin were prepared as described by Volkman *et al.* (1976) and Ohba *et al.* (1977). IgG was isolated from preimmune and hyperimmune antisera by the method described by Harboe and Ingild (1973).

Preparation of cell extracts for RIA. Log phase TN-368-10 cells in 25-cm² Falcon T flasks were inoculated with Ac*M* NPV extracellular virus at a multiplicity of infection (m.o.i.) of 10 to 20. As a control cells were mock infected with TNM-FH medium (Volkman and Sumers, 1976). After 1 hr adsorption with inoculum, TNM-FH medium plus antibiotics (Volkman *et al.*, 1976) were added to the cells. The cells were suspended in the medium by gentle pipetting, transferred to a sterile test tube, and pelleted by centrifugation at 300 *g* for 15 min. The cells were washed twice by gently suspending the pellet in 5.0 ml of the TNM-FH

medium and pelleting by centrifugation. The final cell pellet was suspended in 5 ml of TNM-FH medium. At designated times postinoculation, the cells were dispersed into a homogenous suspension by gentle shaking and pipetting, and samples were removed for analysis. For analysis by RIA cells were counted, adjusted to the appropriate concentration with TNM-FH medium, and pelleted by centrifugation at 300 *g* for 15 min. The cells were washed twice again by gentle suspension in phosphate-buffered saline (PBS), and pelleted. The cell pellet was disrupted by addition of 0.5 ml of 0.1 *M* Na_2CO_3 buffer, pH 10.9, 0.17 *M* NaCl, and 0.01 *M* EDTA. Nonidet P-40 was added to a final concentration of 0.5%. After 30 min at 4° the cellular suspension was sonicated (5-sec-bursts for a total of 30 sec at 4° using a Heat Systems cell disruptor Model 375). The lysate was centrifuged at 300 *g* for 15 min to remove any insoluble cell debris, and the supernatant carefully removed to assay for polyhedrin by RIA.

Immunodiffusion. Immunodiffusion tests were conducted by a modification of the double diffusion method (Ouchterlony, 1958).

Radioimmunoassay. A modification of the micro-solid phase radioimmunoassay described by Purcell *et al.* (1973) was used. Two hundred microliters of 0.05 *M* Na_2CO_3–$NaHCO_3$ buffer, pH 9.6, containing 5 μg of Protein A (Pharmacia Fine Chemicals, Inc.) was placed in flexible polyvinyl microtiter plate wells (Linbro Scientific Co.), incubated at room temperature for 2 hr and then overnight at 4°. The solution was aspirated, each well washed twice with distilled H_2O, and 200 μl of the appropriate dilution of antibody solution added. The antibody solution consisted of 0.01 *M* Tris–HCl, pH 7.5, 0.1 *M* NaCl, 0.001 *M* EDTA, and 0.01% sodium azide (Tris buffer). The microtiter plates were incubated for 8 hr at 4°. The wells were aspirated, washed twice with Tris buffer, and 200 μl of the Tris buffer containing 2% BSA was added to each well to preclude adsorption of antigens to the plastic surface. Following overnight incubation at 4°, the wells were aspirated and 150 μl was pipetted into each well containing 10,000 cpm of ^{125}I-polyhedrin or -granulin in Tris–BSA buffer. The plate was incubated overnight at 4°, the wells were aspirated, washed twice with Tris–BSA buffer, and then three times with the Tris buffer. The microtiter wells were cut from the plate, and assayed for bound ^{125}I in an automatic gamma spectrophotometer (Searle Model 1195).

Competitive inhibition assays were carried out in the same manner except the antibody was used at a dilution which bound 50% of the homologous labeled polyhedrin or granulin. Competing proteins were diluted in the Tris–BSA solution so that each well received 150 μl of the polyhedrin or granulin. After incubation for 24 hr at 4°, 10,000 cpm of ^{125}I-polyhedrin or -granulin (0.01 ml) was added and incubated overnight at 4°. The wells were then washed, cut from the microtiter plates, and counted.

To detect polyhedrin in infected and mock-infected TN-368 cells, the cell lysates described above were diluted in a 0.01 *M* Tris–HCl solution containing 0.01 *M* NaCl, 0.001 *M* EDTA, 0.05% NP-40, and 0.01% NaN_3, pH 7.5. For comparison as a standard cell lysates were prepared with polyhedrin added to known concentrations ranging from 10 pg to 10 μg.

Protein determination. The protein concentration of polyhedrin solutions was determined by the method of Lowry *et al.* (1951) using BSA as a standard.

RESULTS

Purity and Stability of Polyhedrins and Granulins

After inactivation of the protease (Summers and Smith, 1975, 1976; Kozlov *et al.*, 1976; Eppstein and Thoma, 1975) and purification by preparative SDS–polyacrylamide electrophoresis, each polyhedrin and granulin migrated in analytical SDS–polyacrylamide gels as a single band (Fig. 1A) (Summers and Smith, 1978). Polyhedrins and granulins prepared from NPVs and GVs without 70° exposure or $HgCl_2$ pretreatment underwent varying degrees of proteolytic degradation, dependent upon the protein, with time and storage (Fig. 1B). Iodinated Ac*M* NPV polyhedrin (Fig. 1C) and TnGV granulin used in the immunological analysis of polyhedrins and granulins by radioimmunoassay (RIA) migrated in polyacrylamide

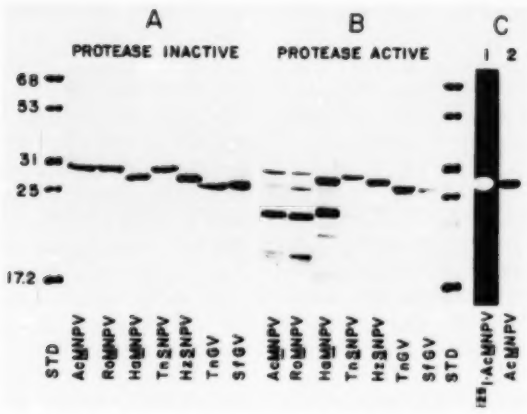

FIG. 1. Analysis of polyhedrins and granulins by analytical polyacrylamide slab gel electrophoresis. (A) Polyhedrins (Ac*M* NPV, Ro*M* NPV, Ha*M* NPV, Tn*S* NPV, Hz*S* NPV) and granulins (TnGV, SfGV) purified by preparative SDS–polyacrylamide gel electrophoresis under conditions of inactive protease as described under Materials and Methods. (B) Polyhedrins and granulins isolated under conditions allowing for active protease. (C) Approximately 20,000 cpm of iodinated Ac*M* NPV polyhedrin in slot C1 and 2.5 μg of unlabeled protein in slot C2 were electrophoresed for 3 hr and stained with Coomassie blue (slot C2). Autoradiography of labeled proteins (slot C1) was performed as described under Materials and Methods. Molecular weights of the standard protein markers are: bovine serum albumin, 68,000; L-glutamic dehydrogenase, 53,000; DNA nuclease, 31,000; chymotrypsinogen, 25,000; and myoglobin, 17,200.

gels as a single band which comigrated with the respective unlabeled polyhedrin, 30,000 MW, (Fig. 1C), or granulin, 25,500 MW, (not shown).

Immunodiffusion of Polyhedrins and Granulins

Several studies report that protease activity does not alter the immunoreactivity of polyhedrins and granulins (Kalmakoff *et al.*, 1978; DiCapua and Norton, 1976; McCarthy and DiCapua, 1979). We decided to conduct a preliminary study to test this and to evaluate the effects of enzyme activity in the standardized RIA.

The immunoreactivity of purified polyhedrins and granulins purified by SDS–PAGE was first analyzed by immunodiffusion (Figs. 2A–F). TnGV granulin reacted with homologous antiserum to form two major and one minor precipitin bands (Fig. 2A). SfGV granulin, possessing a primary structure similar to that of TnGV (Summers and Smith, 1976), showed a reaction of identity with one major TnGV precipitin band. TnGV granulin antiserum also recognized related antigenic determinants in Ac*M* NPV, Ro*M* NPV, Ag*M* NPV, and Tn*S* NPV polyhedrins (Figs. 2B and C). Under the experimental conditions utilized Ha*M* NPV and Hz*S* NPV polyhedrins did not appear to react with TnGV granulin antiserum (Figs. 2B and C). Polyhedrins and granulins degraded by enzyme activity (Fig. 1B) were also reacted with the TnGV granulin antiserum. The immunodiffusion patterns for these proteins and TnGV granulin antiserum are shown in Figs. 2D–F. While immunoreactivity of TnGV granulin with homologous antiserum was maintained the reactions were diminished, for example SfGV, Tn*S* NPV, Ro*M* NPV, and Ag*M* NPV (Figs. 2D–F). In contrast, Ha*M* NPV and Hz*S* NPV polyhedrins show some reaction with TnGV antiserum whereas they did not in preparations where the protease had been inactivated (Figs. 2E and F). Since altered reactions were also detected with Ac*M* NPV polyhedrin antiserum when tested against degraded polyhedrins and granulins (data

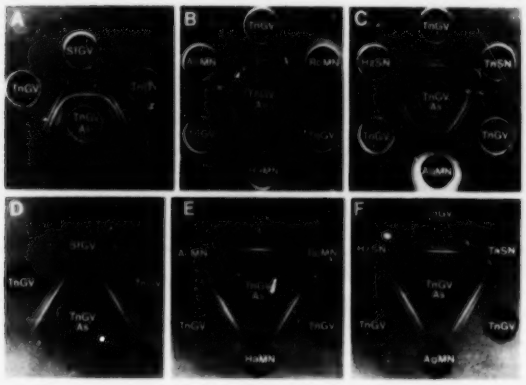

FIG. 2. Immunodiffusion of polyhedrins and granulins. *Trichoplusia ni* granulin antiserum (TnGV As) was reacted with equivalent concentrations (15 μg/well) of undegraded (protease inactive, A–C) and degraded (protease active, D–F) polyhedrins and granulins (see Fig. 1). Plates were incubated for 24 hr at room temperature and photographed directly. The polyhedrins and granulins used were TnGV (*Trichoplusia ni*) granulin; SfGV (*Spodoptera frugiperda*) granulin; AcMN (*Autographa californica*) polyhedrin; RoMN (*Rachiplusia ou*) polyhedrin; HaMN (*Heliothis armigera*) polyhedrin; HzSN (*Heliothis zea*) polyhedrin; TnSN (*Trichoplusia ni*) polyhedrin; AgMN (*Anticarsa gemmatalis*) polyhedrin.

not shown), only undegraded SDS–PAGE-purified antigens were used for RIA.

RIA of AcMNPV Polyhedrin and TnGV Granulin

Titrations of Ac*M* NPV polyhedrin and TnGV granulin antisera with homologous iodinated antigen indicated that dilutions of 1:2600 (Ac*M* NPV As) and 1:6500 (TnGV As) bound 50% of the respective labeled proteins (data not shown). Serial dilutions of unlabeled Ac*M* NPV polyhedrin and TnGV granulin were then employed to competitively inhibit binding of homologous labeled antigen at these antiserum dilutions in order to define assay parameters (Figs. 3A and B). The standard curve for Ac*M* NPV polyhedrin showed that 80 pg of Ac*M* NPV polyhedrin would reproducibly inhibit binding by 10%, establishing the lower limit of sensitivity of the assay (Fig. 3A). Five hundred picograms produced 50% inhibition and 3.0 ng was required for 90% inhibition (Fig. 3A). A minimum detectable quantity of TnGV granulin was 600 pg (Fig. 3B). Fifty percent inhibition of labeled antigen binding was achieved by 7 ng of TnGV granulin while 60 ng resulted in 80% inhibition. Thus, the working range of this assay was 600 pg to 60 ng (Fig. 3B).

RIA of AcMNPV Polyhedrin Synthesized in Vivo

The immunoreactivity of Ac*M* NPV polyhedrin purified in the presence of SDS to homologous antiserum was compared with the immunoreactivity of polyhedrin produced in infected cells. Figure 4 shows reaction curves obtained by competing 10-fold dilutions of lysed infected TN-368 cells. The competition reaction curve parallels a standard curve obtained by adding known amounts of SDS-purified Ac*M* NPV polyhedrin to uninfected cell lysates. The linear regions of each curve gave essentially identical slopes, indicating that the antigenic determinants of SDS-purified polyhedrin and polyhedrin synthesized in infected cells had a very similar immunoreactivity with this antiserum. Standard curves prepared with highly purified Ac*M* NPV polyhedrin in buffer also demonstrated identical slopes (data not shown). However, the sensitivity of the standard assay in the absence of cell lysate was approximately 5-fold greater (80 pg to 3 ng), indicating some interference with

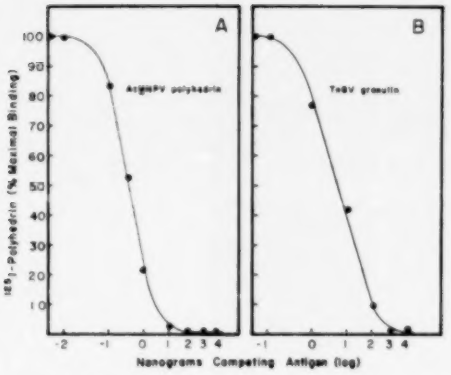

FIG. 3. Radioimmunoassay of Ac*M* NPV polyhedrin and TnGV granulin. Tenfold dilutions of Ac*M* NPV polyhedrin (A) and TnGV granulin (B) in quantities from 10 pg to 10 μg were reacted for 24 hr with that dilution of homologous antiserum which bound 50% of homologous iodinated antigen. In each homologous assay 10,000 cpm of ^{125}I-Ac*M* NPV polyhedrin or ^{125}I-TnGV granulin was added to each well, incubated for 24 hr, the wells were washed, and the percentage maximal binding was determined as described under Materials and Methods.

assay performance in the presence of the cellular lysate.

Competitive Inhibition Studies of Polyhedrins and Granulins

Since studies with Ac*M* NPV polyhedrin *in vivo* did not show altered immunoreactivity of SDS–PAGE-purified polyhedrin with antibody, a comparison was made of four polyhedrins and two granulins using competitive inhibition of ^{125}I-Ac*M* NPV polyhedrin binding to homologous antiserum. The results (Figs. 5A and B) show that all polyhedrins and granulins assayed contain antigenic determinants similar to determinants of Ac*M* NPV polyhedrin recognized by homologous antiserum. However, either the affinity of those determinants varied among the proteins studied, or the number of determinants, or both. While 0.5 ng of Ac*M* NPV polyhedrin inhibited the binding of ^{125}I-Ac*M* NPV polyhedrin by 50%, 5.0, 8.0, and 200 ng of Ro*M* NPV, Tn*S* NPV, and Ha*M* NPV polyhedrins, respectively, was required to achieve equivalent inhibition (Fig. 5A). Fifty percent inhibition with TnGV and SfGV granulins and Hz*S* NPV polyhedrin required 75, 85, and 150 ng, respectively (Fig. 5B). Also, the slopes of the inhibition curves for heterologous polyhedrin and granulins indicated that Ac*M* NPV polyhedrin antiserum did not bind to those proteins with comparable ability.

A similar competition experiment with anti-TnGV granulin serum against ^{125}I-TnGV granulin produced the reaction curves shown in Figs. 6A and B. Seven nanograms of the homologous antigen was required to competitively inhibit by 50% the binding of ^{125}I-TnGV granulin to antigranulin serum. For

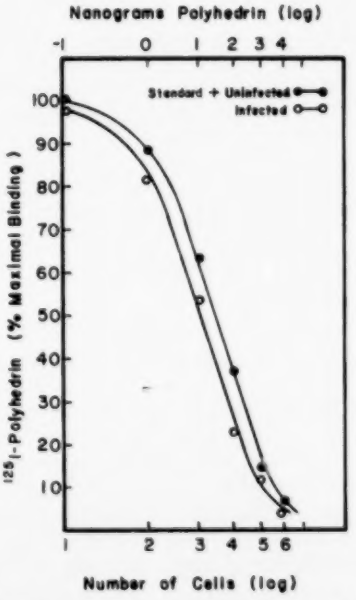

FIG. 4. Detection of Ac*M* NPV polyhedrin in infected cells. Tenfold dilutions of infected TN-368 cell lysates (○) starting with 10^6 cells were prepared as described under Materials and Methods and compared for ability to react with Ac*M* NPV polyhedrin antiserum at the dilution which bound 50% of the iodinated Ac*M* NPV polyhedrin indicator. Mock-infected cell lysates (●) from 10^6 cells diluted in a similar manner containing 10 μg to 10 pg of highly purified Ac*M* NPV polyhedrin were added, the incubation was continued for 24 hr, and percentage maximal binding was determined. In a parallel study we were able to detect 1 ng of Ac*M* NPV polyhedrin at 12 hr postinfection in 10^3 cells.

comparison, 9.5 ng of SfGV granulin, 40 ng of Ac*M* NPV polyhedrin, and 400 ng of Tn*S* NPV polyhedrin were necessary to produce 50% inhibition (Fig. 6A). Ro*M* NPV, Hz*S* NPV, and Ha*M* NPV polyhedrins required 45 ng, 330 ng, and 4 μg, respectively, for the same level of inhibition (Fig. 6B). The possibility of additional inhibition by Tn*S* NPV, Hz*S* NPV, and Ha*M* NPV polyhedrins was not tested since the upper limit of the assay was 10 μg. Of note is the failure of SfGV granulin to effect complete inhibition of ^{125}I-TnGV granulin at high antigen concentrations (Fig. 5A).

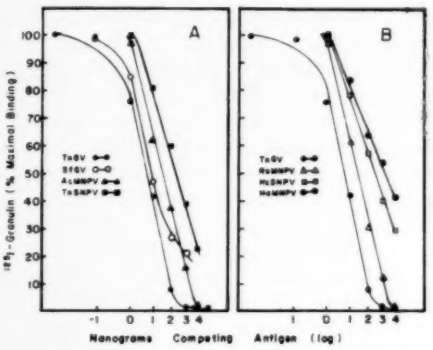

FIG. 6. Comparison of polyhedrins and granulins by RIA utilizing TnGV granulin antiserum. Tenfold dilutions of purified polyhedrins and granulins ranging from 10 μg to 10 pg were tested for the ability to inhibit the binding of 10,000 cpm of ^{125}I-TnGV granulin to homologous antiserum. The results are expressed as percentage maximal binding in the absence of competing antigen. The polyhedrins and granulins used were: TnGV granulin (●); SfGV granulin (○); Ac*M* NPV polyhedrin (▲); Tn*S* NPV polyhedrin (■); Ro*M* NPV polyhedrin (△); Hz*S* NPV polyhedrin (□); and Ha*M* NPV polyhedrin (⊙).

DISCUSSION

This study documents the successful utilization of preparative SDS–polyacrylamide gel electrophoresis to isolate and purify sufficient quantities of a major baculovirus structural protein for immunological studies. We have been able to show that antiserum raised against Ac*M* NPV polyhedrin purified in the presence of SDS recognized polyhedrin translated in infected cells and the SDS-treated standard with similar ability (Fig. 4). Our data support results published with vertebrate viruses showing that the immunological integrity and specificity of certain viral proteins are retained when isolated in the presence of SDS (Yagi *et al.*, 1978; Lane and Robbins, 1978; Carroll *et al.*, 1978).

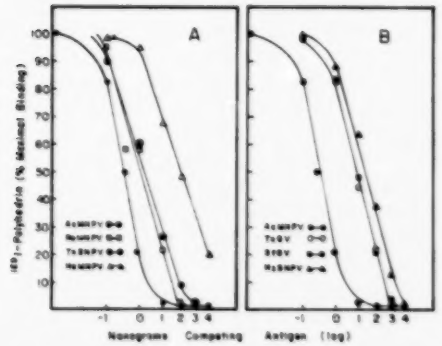

FIG. 5. Comparison of polyhedrins and granulins by RIA. Tenfold dilutions of purified polyhedrins and granulins ranging from 10 μg to 10 pg were reacted with the dilution of Ac*M* NPV polyhedrin antiserum which would bind 50% of iodinated homologous antigen. After incubation for 24 hr, 10,000 cpm of ^{125}I-Ac*M* NPV polyhedrin was added, and the ability of competing proteins to inhibit binding was determined and expressed as percentage maximal binding in the absence of competing antigen. The competing antigens used were: Ac*M* NPV polyhedrin (●); Ro*M* NPV polyhedrin (□); Tn*S* NPV polyhedrin (⊙); Ha*M* NPV polyhedrin (△); TnGV granulin (○—○); SfGV granulin (■); and Hz*S* NPV polyhedrin (▲).

Among the basic problems associated with the serology of baculoviruses and baculovirus proteins are antigen purity (Bell and Orlob, 1977; Harrap *et al.*, 1977; Summers and Smith, 1978) and potential effects as a result of degradation by an inclusion-associated protease (Eppstein and Thoma, 1975; Kozlov *et al.*, 1975; Summers and Smith, 1975, 1976). In our hands altered immunoreactivity of protease-degraded polyhedrins or granulins was detected as altered immunodiffusion reactions (Figs. 2D–F) and reduced sensitivity and immunoreactivity in RIA (data not shown). Previous studies which used protease active preparations of polyhedrins (DiCapua and Norton, 1979) did

not detect changes in the immunoreactivity or specificity of polyhedrins when assayed by immunodiffusion. However, the degree of enzyme hydrolysis and the purity and physical properties of the proteins used for those studies were not determined. Inactivation of the protease and the use of SDS–polyacrylamide gel-purified antigens for antisera production and immunological analysis alleviate this source of potential artifact.

Results obtained in the immunodiffusion comparisons of SDS–PAGE-purified proteins reveal that at least three different antigenic determinants are present on TnGV granulin (Fig. 2A). Since the granulins and polyhedrins are single polypeptides (Fig. 1), the multiple precipitin arcs in immunodiffusion of TnGV granulin are probably the result of aggregation of granulin monomers into higher molecular weight complexes. Complex formation could then induce a tertiary antigenic determinant recognized by a different antibody population in the antiserum. Recently, Rohrmann (1977) has shown that alkali-dissolved polyhedrins from two NPVs of *Orgyia pseudotsugata* were composed of a 12 S molecule of 209,000 MW which, upon reduction with mercaptoethanol, was believed to contain eight subunits each of approximately 26,000 MW. The results of those immunodiffusion studies suggest that both the subunit and the 12 S molecule contained separate antigens, and that those antigens cross-reacted between the two polyhedrins studied. Our results show that only one of the TnGV granulin antigens cross-reacts with other polyhedrins and granulins tested. Whether this antigen may represent an antigenic determinant on the subunit or a higher molecular weight complex is unknown at present.

Two-dimensional high voltage electrophoresis of tryptic peptides from polyhedrin and granulin proteins has shown that all proteins analyzed contain some tryptic peptides with similar size and electrophoretic mobility. The number of similar peptides varies when comparing different proteins (Summers and Smith, 1976; Maruniak and Summers, 1978). Yet each polyhedrin or granulin contains tryptic peptides which are unique. TnGV and SfGV granulins are very similar and this is confirmed by both immunodiffusion (Fig. 2A) and competitive inhibition studies (Fig. 6). Polyhedrins considered to be less similar by comparing tryptic peptides (Maruniak and Summers, 1978) also form a larger spur in immunodiffusion with TnGV granulin (Figs. 2B and C). This indicates that common antigenic determinants exist on all the polyhedrins and granulins compared but some proteins, in this case TnGV granulin, have additional antigenic determinants. Similar results were not obtained using anti Ac*M* NPV polyhedrin serum.

Our results from RIA aid in further characterizing the "group antigen" of polyhedrins and granulins. Polyhedrins and granulins were shown to be heterologous with respect to their ability to compete with the homologous antigen in the assay. For example, Ha*M* NPV and Hz*S* NPV polyhedrin were not observed to react with TnGV granulin antiserum in the immunodiffusion assay (Figs. 2B and C). When 500- and 100-fold greater concentrations of these proteins were compared by RIA, inhibition of binding to homologous serum occurred (Figs. 5 and 6). Thus, the more sensitive immunological assay could measure immunological relatedness of Ha*M* NPV and Hz*S* NPV not detected by immunodiffusion. Care must be taken, however, in making quantitative comparisons of the immunoreactivity of polyhedrin and granulin, since antigenic determinants were revealed in Ha*M* NPV and Hz*S* NPV polyhedrins by enzyme hydrolysis which are apparently not accessible to antibody in the undegraded molecule (Figs. 2E and F). Also, aggregation of polyhedrin or granulin to higher molecular weight complexes may eliminate or modify antigenic determinants formerly accessible to antibody.

In addition to quantitative differences in polyhedrin and granulin "group antigens," qualitative differences in the structure of antigenic determinants were shown to exist. This is suggested by the reduction in slopes of heterologous competition curves, indicating altered binding ability for antibody sites as compared to the homologous antigen (Figs. 5 and 6). However, because of the

high dilution of antiserum in competition RIA, the specific TnGV antigenic determinant detected by immunodiffusion may be lost.

Crawford *et al.* (1977, 1978) emphasized the use of RIA primarily as a means of detecting baculoviruses in environmental studies. They have not utilized antisera prepared against highly purified polyhedrins, nor have they utilized competitive RIA as a tool to compare and analyze the serology of polyhedrins as antigens. The data obtained for competition RIA herein provide new information on the serological properties of this class of major baculovirus structural proteins. Because of the serological similarities identified among apparently closely and distantly related baculoviruses shown in this study, it will be difficult to use the present application for identification. Emphasis should be given to other baculovirus structural polypeptides for developing RIA for serological comparisons. The sensitivity and specificity of RIA will aid in the study of baculovirus structural proteins relative to their identity and role in biological specificity.

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